Excessive cycling converts PCR products to random-length higher molecular weight fragments

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During the development of a method to sequence PCR products from cell lysates of Salmonella *hisD3052* revertants generated in the Ames mutagenicity assay (1), we wondered why PCR reactions greater than 30 cycles often resulted in reduced amounts of specific product and also produced smears on agarose gels.

To investigate this effect, two 100-μl PCR reactions, identical except for the primer concentrations (50-pmol or 100-pmol), were performed as described below, and 5-μl aliquots were removed after 20, 26, 32, 38, and 44 cycles. These aliquots were electrophoresed on a 1% agarose/2% NuSieve GTG agarose gel, Southern blotted to nitrocellulose, and hybridized with a $^{[32]}$P-endlabeled oligonucleotide probe by standard methods. The results are displayed in lanes 6–15 of Figure 1a and 1b. Maximum amplification of the 328-bp product in the two parallel reactions occurred at about 26 cycles. Upon further cycling, specific product yield was reduced, additional bands appeared, and a smear developed. As can be seen in lane 10, the specific product has nearly disappeared after 44 cycles. The Southern blot hybridization also shows this effect and demonstrates that the smear contains sequences internal to the specific PCR fragment. Comparison of the higher cycles of the 50-pmol reaction to those of the 100-pmol reaction (lanes 9, 10 and 14, 15) reveals that in the latter case the loss of product has not progressed to the same extent. When a 60°C annealing temperature for 36 cycles was used (lane 2, 5°C higher than lanes 6–15), the smearing was eliminated.

These results suggest that after ~30 cycles, most of the PCR primers have been converted into PCR product, and that at this time reaction conditions appear to favor the annealing of the 3′-OH ends of the PCR product to genomic template or to itself. The 3′-OH ends of the PCR product are then extended to higher molecular weight DNA and are randomly terminated during the additional cycles. These random-length products are the likely components of the smear observed on the agarose gel and Southern blot. Upon further cycling, the intensity of the smear increases as the intensity of the specific 328-bp band decreases. In the 100-pmol reaction, the conversion process is delayed because sufficient primers are still available. The lack of nonspecific product in lane 2 suggests that the higher annealing temperature (60°C vs 55°C) completely blocks this conversion process.

It is generally recommended that the total number of cycles in PCR be minimized to avoid the buildup of nonspecific products. The results presented here support this recommendation and also demonstrate that excessive cycling can lead to a dramatic loss of specific PCR product.

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REFERENCE

1. Bell,D.A., Levine,J.G. and DeMarini,D.M. (1991) *Mutat. Res.* **252**, 35–44.

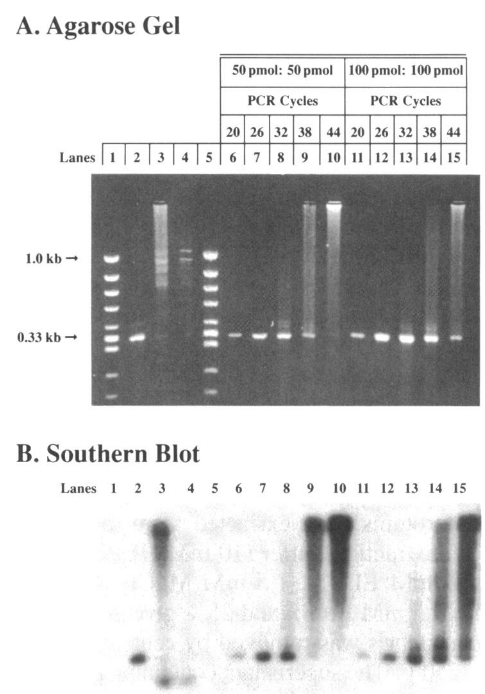

Figure 1. The effect of excessive cycling on PCR yield is shown in lanes 6–15 (see text for explanation). PCR conditions were 94°C for 3 min; multiple cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min as indicated in the lane headings; 72°C for 5 min. Reactions contained 16.6 mM $(NH_4)_2SO_4$, 50 mM β-mercaptoethanol, 6.8 μM EDTA, 67 mM Tris (pH 8.8), 80 μg/ml BSA, and 6.7 mM $MgCl_2$, 200 μM dNTPs, 50 pmol of each primer, 1.25 units of Taq polymerase (*Amplitaq*, Perkin-Elmer Cetus), 5% DMSO (v/v) and ~10 ng of Salmonella DNA. Lanes 1 and 5 contained 1 μg of ΦX174 cut by *Hinc*II. PCR reactions contained in lane 2, 50 pmol each primer, 36 cycles, 60°C annealing temperature; in lanes 6–10, 50 pmol each primer, cycles as indicated; in lanes 11–15, 100 pmol each primer, cycles as indicated.